INTRODUCTION

The contribution of methane (CH₄) to global warming is estimated to be about 20% (IPCC, 2007). Enormous quantities of carbon occur in marine sediments in the form of CH₄ (Kvenvolden, 1988) and at least some CH₄ in marine sediments seeps into the ocean and thus into the atmosphere (Etiope and Milkov, 2004; Judd, 2004; Mau et al., 2007). The mechanisms and fate of this seeping as well as its quantification are poorly constrained. In particular, CH₄ emitted from deep seafloor sources has been ignored to date in estimating atmospheric CH₄ budgets (Etiope and Milkov, 2004; Judd, 2004). Indeed, most CH₄ would be oxidized within bottom water if vertical eddy diffusion in the water column is the only process for transporting CH₄ from water depths greater than 2000 m to surface (Merewether et al., 1985; Paull et al., 1995; Heesch et al., 2003; Sauter et al., 2006). In addition, episodic huge discharges of CH₄ due to massive decomposition of
Seafloor methane hydrates have been suggested as possible causes of sudden climate changes in the geological past (MacDonald, 1990; Dickens et al., 1997; Bains et al., 1999; Svensen et al., 2004). Besides having environmental significance, seafloor CH$_4$ bubbles might also be a possible geohazard and have a resource significance (Sills and Wheeler, 1992).

In 1997, gas bubbling with large-scale chemosynthetic communities and carbonate crusts including chimney-like structures were found on top of Kuroshima Knoll (depth of ca. 640 m), located in the forearc region of the Ryukyu island arc (Matsumoto et al., 1998) (Figs. 1 and 2). Subsequent studies revealed that the bubbles were composed mostly of CH$_4$ (Machiyama et al., 2001). Based on both $\delta^{13}$C and $\delta^{18}$O values of the carbonate crusts, Takeuchi et al. (2007) concluded that methane hydrate layers had developed under the area. The CH$_4$-rich fluid derived from the partial dissociation of the methane hydrate produced both the bubbles and the carbonate crusts (Takeuchi et al., 2007). They assumed that subtle changes in the marine environment were responsible for the dissociation of methane hydrate. If this assumption is correct, the area is an important field for studying the dissociation process for seafloor methane hydrate and discussing future possible changes in seafloor CH$_4$ flux to the ocean and atmosphere. However, we had little evidence for the existence of methane hydrate beneath the seafloor of the knoll and for the progression of dissociation. In addition, the influence of CH$_4$ discharge on the surrounding marine environment was poorly constrained.

In this study, we collected samples of both gas bubbles and bottom fluid from the active gas seeping site of Kuroshima Knoll (Fig. 2) using the WHATS gas-tight sampler (Water and Hydrothermal-fluid Atsuryoku Tight

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Fig. 1. Locations of Kuroshima Knoll (square), Taketomi spring (circle), and submarine Quaternary volcanic features (▲) (modified from Machiyama et al., 2001). The detailed bottom topography in the square (the area around Kuroshima Knoll) is presented in Fig. 2.
attached to the vehicle Shinkai 2000 to determine the origin of CH₄ and discuss the possible sub-seafloor dissociation of methane hydrate using geochemical tracers. Knowledge of the origin of CH₄ (i.e., biogenic from acetate fermentation or CO₂ reduction, thermogenic, inorganic) and the other coexisting gases would provide information on the processes and environments responsible for their generation. Thus seepage gases could be used as a tracer for sub-seafloor hydrocarbon reservoirs and of secondary processes, such as migration through geodynamic processes (Price and Schoell, 1995; Etiope et al., 2009), microbial alteration (Head et al., 2003; Kinnaman et al., 2007), and gas hydrate formation/decomposition (Sassen et al., 2001; Milkov et al., 2004).

Besides the gas bubbles and seeping fluid at the seafloor, the effluent plume in the water column was sampled in and around the knoll to evaluate the distribution and the fate of the CH₄ bubbles in the ocean water column, with special emphasis on the influence of aerobic CH₄ oxidation on the fate of CH₄ in the water column. The distribution of the concentration and stable carbon isotopic composition (δ¹³C) of CH₄ in a CH₄-enriched plume are good tracers for determining the fate of CH₄ in a water column, especially for quantitatively determining the progress of microbial oxidation (Tsunogai et al., 1998, 2000, 2005; Nishimura et al., 1999). Indeed, CH₄ oxidation consumes ¹³CH₄ preferentially, leaving the residual CH₄ enriched in ¹²CH₄ (Silverman and Oyama, 1968), while δ¹³C(CH₄) does not vary through eddy diffusion. In addition, it was shown that the kinetic isotope effect (KIE) α is around 1.005 at the bottom of the water column (Tsunogai et al., 2000). α is defined as the ratio of the relative reaction rates of molecules containing different isotopes:

\[ \alpha = k_{13} / k_{12}, \]  

(1)

where \( k_{13} \) and \( k_{12} \) are the respective rate constants for

\[ ^{13}\text{CH}_4 + 2\text{O}_2 \rightarrow ^{13}\text{CO}_2 + 2\text{H}_2\text{O} \]  

(2)

and

\[ ^{12}\text{CH}_4 + 2\text{O}_2 \rightarrow ^{12}\text{CO}_2 + 2\text{H}_2\text{O}. \]  

(3)
Using the distribution of $\delta^{13}C_{CH}$ in a CH$_4$-enriched plume and the determined KIE, we traced the progress of CH$_4$ oxidation in the CH$_4$-enriched plume and determined whether the main cause of the CH$_4$ decrease in the water column was eddy diffusion (dilution) or oxidation.

**GEOLOGICAL BACKGROUND**

The Kuroshima Knoll (Figs. 1 and 2) is about 26 km south of the island of Ishigaki in the forearc region of the southern Ryukyu arc. The knoll has a flat table for its top at a depth of 1000 to 630 m, while the foot part reaches to a depth of 2400 m. On the basis of geological observations on the mudstones, sandstones, and mud cores recovered from the knoll, Cenozoic sediments, such as the Pliocene Shimajiri Group, are assumed to cover most of its surface (Matsumoto et al., 1998; Takeuchi et al., 2007).

The Cenozoic of the Ryukyu arc is divided into the lower Eocene Wano and Kayo Formations, upper Eocene Miyara Group, lower Miocene Yaeyama Group, Sonai conglomerate of unknown age, middle Miocene Takarajima Group, Pliocene Shimajiri Group, middle to upper Pleistocene Ryukyu Group and Holocene coastal and terrestrial deposits (Nakagawa, 1983). The region of the southern Ryukyu arc, including the islands of Ishigaki and Irionmote (Fig. 1), can be characterized by volcanic activity in the Miocene, such as the 21 Ma old granitic intrusions in Ishigaki (Kawano and Ueda, 1966) and volcanic breccia, tuff breccia, and lava flows along with small dikes in Irionmote (Monden, 1968; Tiba and Saito, 1974). On the other hand, the Pliocene Shimajiri Group consists mostly of fossiliferous sandstone and siltstone. Except for the backarc region (Fig. 1), no volcanic activity has been found in the southern Ryukyu forearc area from the Pliocene to the present (Nakagawa, 1983). Large-scale colonies of Calyptogena kawamurai and Bathymodiolus spp. (both living and dead) (Okutani et al., 2004), calcareous crusts, many gravels of sand chimneys, and emissions of gas bubbles have been found on the seafloor in diving surveys (Matsumoto et al., 1998; Machiyama et al., 2001). Active microbial oxidation of CH$_4$ (both aerobic and anaerobic) has been found in sediments just beneath the seafloor, especially in areas that can be characterized by slow, diffusive seeping of CH$_4$ gas or CH$_4$-enriched fluid (Inagaki et al., 2004).

On the basis of the $^{13}$C-depleted carbon isotopic ratios (from $-4.1$ to $-43.2$‰ PDB) and the $^{18}$O-enrichment (from $+2.5$ to $+7.9$‰ PDB) of the carbonates, Takeuchi et al. (2007) proposed that methane hydrate layers could have developed under Kuroshima Knoll. The CH$_4$-rich,
Table 2. Contents and stable carbon isotopic compositions of CH₄ in the bottom fluid samples, together with their sampling locations, depths, and heights from the seafloor

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Latitude (N)</th>
<th>Longitude (E)</th>
<th>Depth* (m)</th>
<th>Height** (m)</th>
<th>CH₄ (µmol/kg)</th>
<th>δ¹³Cᵥ (‰ vs VPDB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples taken by WHATS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1355W2</td>
<td>24°07.798'</td>
<td>124°11.547'</td>
<td>638</td>
<td>&lt;0.3</td>
<td>52</td>
<td>−33.3</td>
</tr>
<tr>
<td>D1355W4</td>
<td>24°07.798'</td>
<td>124°11.565'</td>
<td>641</td>
<td>&lt;0.3</td>
<td>19</td>
<td>−37.0</td>
</tr>
<tr>
<td>D1356W2</td>
<td>24°07.805'</td>
<td>124°11.540'</td>
<td>637</td>
<td>&lt;0.3</td>
<td>2.0 × 10⁴</td>
<td>−33.0</td>
</tr>
<tr>
<td>D1356W3</td>
<td>24°07.831'</td>
<td>124°11.553'</td>
<td>638</td>
<td>&lt;0.3</td>
<td>1.5 × 10⁴</td>
<td>−37.5</td>
</tr>
<tr>
<td>D1357W2</td>
<td>24°07.800'</td>
<td>124°11.536'</td>
<td>638</td>
<td>&lt;0.3</td>
<td>662</td>
<td>−35.0</td>
</tr>
<tr>
<td>D1357W3</td>
<td>24°07.800'</td>
<td>124°11.536'</td>
<td>638</td>
<td>&lt;0.3</td>
<td>1.4 × 10⁴</td>
<td>−37.8</td>
</tr>
<tr>
<td>D1357W4</td>
<td>24°07.810'</td>
<td>124°11.571'</td>
<td>641</td>
<td>&lt;0.3</td>
<td>2.3 × 10⁴</td>
<td>−38.3</td>
</tr>
<tr>
<td>D1360W2</td>
<td>24°07.811'</td>
<td>124°11.134'</td>
<td>643</td>
<td>&lt;0.3</td>
<td>1.1</td>
<td>−39.8</td>
</tr>
<tr>
<td>D1363W2</td>
<td>24°07.805'</td>
<td>124°11.535'</td>
<td>636</td>
<td>&lt;0.3</td>
<td>601</td>
<td>−33.7</td>
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<tr>
<td>D1363W4</td>
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<td>124°11.532'</td>
<td>635</td>
<td>&lt;0.3</td>
<td>55</td>
<td>−39.8</td>
</tr>
<tr>
<td>D1364W2</td>
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<td>642</td>
<td>&lt;0.3</td>
<td>13</td>
<td>−31.3</td>
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<tr>
<td>D1364W4</td>
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<td>124°11.129'</td>
<td>642</td>
<td>&lt;0.3</td>
<td>1.5</td>
<td>−40.4</td>
</tr>
<tr>
<td>D1366W2</td>
<td>24°07.814'</td>
<td>124°11.346'</td>
<td>635</td>
<td>&lt;0.3</td>
<td>3.3 × 10⁴</td>
<td>−32.2</td>
</tr>
<tr>
<td>D1368W4</td>
<td>24°07.815'</td>
<td>124°11.352'</td>
<td>640</td>
<td>&lt;0.3</td>
<td>165</td>
<td>−40.3</td>
</tr>
<tr>
<td>Samples taken by Niskin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1355N2</td>
<td>24°07.798'</td>
<td>124°11.547'</td>
<td>638</td>
<td>1.5</td>
<td>12</td>
<td>−38.2</td>
</tr>
<tr>
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<td>124°11.553'</td>
<td>638</td>
<td>1.5</td>
<td>1.3</td>
<td>−37.4</td>
</tr>
<tr>
<td>D1360N1</td>
<td>24°07.772'</td>
<td>124°11.527'</td>
<td>637</td>
<td>1.5</td>
<td>0.9</td>
<td>−38.7</td>
</tr>
<tr>
<td>D1360N2</td>
<td>24°07.814'</td>
<td>124°11.535'</td>
<td>636</td>
<td>1.5</td>
<td>0.9</td>
<td>−38.7</td>
</tr>
<tr>
<td>D1363N2</td>
<td>24°07.805'</td>
<td>124°11.535'</td>
<td>636</td>
<td>1.5</td>
<td>63</td>
<td>−36.1</td>
</tr>
</tbody>
</table>

*The seafloor depth.
**Approximate sampling height from the seafloor.

**Approximate sampling height from the seafloor.

18O-enriched fluids likely derived from the partial dissociation of the methane hydrate produced both the bubbles and carbonates on the seafloor (Takeuchi et al., 2007). Because methane hydrate is theoretically stable in the top 70 m layer of the knoll, Takeuchi et al. (2007) assumed some subtle changes in the marine environment as possible cause of the dissociation of methane hydrate. Other than the δ¹⁸O values of the carbonates, however, there is little evidence for the existence of methane hydrate beneath the seafloor of the knoll.

**Sampling**

Gas bubbles and bottom fluid (seawater taken just above the seafloor of the gas seepage site) were collected by the human occupied vehicle Shinkai 2000 of the Japan Agency for Marine-Earth Science and Technology (JAMSTEC) during the NT02-08 cruise in June 2002, using the WHATS (Tsunogas et al., 2003; Saegusa et al., 2006). The sampler comprised four 150 cm³ stainless steel sample cylinders, eight ball valves, a motor-driven arm, a rail, a peristaltic pump, and a flexible Teflon tube connected to the inlet. The motor-driven arm on the rail is used to open one of the four cylinders. By pumping out seawater that had filled the cylinder, we introduced sample fluid/gas from the inlet into the cylinder. After completely replacing the seawater with sample, the valves at both ends of the cylinder are tightly closed by moving the motor-driven arm again. At this stage, the next cylinder is ready for sampling. The entire operation was conducted inside the submersible cabin.

In dives for sampling bottom fluid, a titanium inlet tube was used for the sample inlet, while a specially designed funnel-shaped inlet was used for sampling gas bubbles (Saegusa et al., 2006). Because we could not recognize fluid seeping from Kuroshima Knoll, we took seawater less than 30 cm above the seafloor, in areas where active fluid seeping was anticipated, such as areas covered by dense Bathymodiolus mussels. A temperature probe was attached to the titanium inlet tube to measure the temperature of the incoming bottom fluid. The bottom fluid samples, however, showed temperatures close to that of the ambient seawater (about 8.0°C), so that we could not clarify the difference from the ambient seawater. In addition to samples taken by the WHATS, samples of gas bubbles were collected by non-gas-tight, cylinder
samplers made of acrylic resin (noted as M-type samplers; Sakai et al., 1990; Gamo et al., 1991) and samples of bottom fluid were collected by Niskin samplers. The Niskin samplers were attached to the Shinkai 2000 and operated at the heights of 1.5 m from the seafloor. All the samples of gas bubbles and bottom fluid are listed in Tables 1 and 2, respectively.

The collected samples were immediately subsampled into several kinds of bottles in the onboard laboratory of the mother ship Natsushima. For the determinations of the major components in the gas bubbles (N₂, O₂, H₂S, CH₄, CO₂, CO, and non-methane hydrocarbons), each sample in a stainless steel cylinder was expanded into a container (50 cm³) made of stainless steel with a ball valve at each end (Xu et al., 1995) and stored under inner pressures higher than atmospheric pressure. In addition, for the determination of noble gases in the bubbles, an aliquot of gas sample was expanded into a pre-evacuated line and subsampled in a lead glass bottle (50 cm³) under a pressure slightly higher than atmospheric pressure.

For measurements of dissolved gas components, especially CH₄, in the samples of bottom fluid, each fluid sample taken in a stainless steel cylinder was first transferred into a pre-evacuated line (ca. 1,500 cm³ volume) and mixed with both mercury chloride (to remove dissolved H₂S as HgS) and phosphoric acid (to reduce the pH of the fluid to less than 1). After waiting for gas exchange equilibrium between the gas and the liquid phase within the line, the pressure was measured for the gas phase and part of it was subsampled into a container (50 cm³) made of stainless steel with a bellows valve at each end.

Each sample of gas bubbles collected by the M-type bottom sampler was subsampled into a glass bottle (200 cm³) with a stopcock at each end through the downward displacement of pure water. Each sample of ambient bottom seawater collected by the Niskin sampler was slowly transferred into 65 cm³ glass vials, for the determination of the concentration and δ¹³C of CH₄ (Tsunogai et al., 2000). After approximately 3-fold volume overflow to prevent air contamination, 0.5 cm³ of saturated HgCl₂ solution (6 wt%) was slowly added to each vial as a preservative. To minimize air contamination with the addition of HgCl₂ solution, the solution was degassed immediately prior to its addition. The vial was then sealed with a butyl rubber stopper and stored in the dark at 4°C until analysis. The actual sampling depth of each Niskin bottle was verified by analyzing the salinity and dissolved oxygen concentration for each bottle using standard methods (Carpenter, 1965).

**ANALYTICAL METHODS**

The concentrations and δ¹³C values of CO₂, CH₄, CO and non-methane hydrocarbons in the gas samples (including the gas phase samples extracted from the bottom fluid samples) were determined by continuous-flow isotope ratio mass spectrometry (CF-IRMS) at Hokkaido University (Tsunogai et al., 1999, 2000, 2002b; Ijiri et al., 2003; Komatsu et al., 2005). The concentrations of N₂, O₂, H₂, and H₂S in the gas samples were determined...
through conventional methods (Sakai et al., 1976). The helium and neon contents and $^{3}$He/$^{4}$He ratio in the gas samples were measured with a modified-VG5400 (MS-III) noble gas mass spectrometer in the University of Tokyo with an accuracy better than 4%, following the procedure described by Aka et al. (2001), except for using the interlaboratory helium standard (HESJ, Matsuda et al., 2002) for the standard material of the $^{3}$He/$^{4}$He ratio.

**RESULTS**

**Gas bubbles**

Analytical results for the bubble samples are listed in Table 1 and presented in Figs. 3, 4, and 5. The samples of gas bubbles taken by the WHATS (D1365W1, D1365W2, W1365W3, and D1367W2 in Table 1) were composed mostly of CH$_4$ (C$_1$) having uniform $\delta^{13}$C values around $-40$‰ VPDB while the concentrations of N$_2$, O$_2$, and H$_2$S were small, less than 1 vol%. Only trace amounts of CO$_2$ and CO were detected; less than 100 ppmv and less than 3 ppmv respectively. Non-methane hydrocarbons were also present in trace amounts, having concentrations of 300 ± 20 ppmv for ethane (C$_2$), 21 ± 14 ppmv for propane (C$_3$), 1.6 ± 0.9 ppmv for isobutane (iso-C$_4$), 1.0 ± 0.5 ppmv for n-butane (n-C$_4$), 0.4 ± 0.04 ppmv for isopentane (iso-C$_5$), and 0.02 ± 0.002 ppmv for n-pentane (n-C$_5$). The C$_4$/(C$_2$+C$_3$) ratio is greater than 3400 (Fig. 3) and the C$_2$/C$_3$ ratio is greater than 9. The $\delta^{13}$C values of the non-methane hydrocarbons were characterized by $^{13}$C enrichment relative to C$_1$, which gave $-28.3^{\circ}$VPDB for C$_2$, $-19.1^{\circ}$±$2.2^{\circ}$VPDB for C$_3$, $-22.4^{\circ}$±$0.2^{\circ}$VPDB for n-C$_4$, $-19.9^{\circ}$±$2.0^{\circ}$VPDB for iso-C$_4$, $-28.5^{\circ}$±$0.4^{\circ}$VPDB for n-C$_5$, and $-16.9^{\circ}$±$1.5^{\circ}$VPDB for iso-C$_5$ (Fig. 5). Noble gases in the samples were characterized by both helium enrichment (9.6 to 13.1 ppmv) and high $^{4}$He/$^{20}$Ne ratios (from 15 to 545) in comparison with values for air, indicating that no air contamination or fractionation affected bubble samples taken by the WHATS. Thus the concentration and isotopic composition of gases represented those of the bubbles on the seafloor. On the other hand, the gas samples taken by the M-type sampler (D1357M2 and D1364M1 in Table 1) were rather different from those taken by the WHATS, having concentrations for C$_1$, N$_2$, and O$_2$ of 20 vol%, 55
vol% and 25 vol%, respectively. We concluded that air had significantly contaminated the samples, presumably during the transportation from the seafloor to the surface. Thus, we do not consider them in the discussions that follow. To determine the concentration and isotopic composition of a gas sample taken from a deep seafloor, the sample should be thus kept gas-tight during transportation.

**Bottom fluid**

Analytical results for the bottom fluid samples, which might represent a mixture of bottom seawater and seeping material in the knoll, are presented in Table 2. They show significant enrichment in CH$_4$, especially for samples taken just above the seafloor (with sampling heights less than 0.3 m from the seafloor) by the WHATS. The observed concentration of CH$_4$ almost corresponds to the concentration under a saturation condition of about 10$^5$ µmol/kg for the pressure and the temperature at the seafloor depth (65 bar and 8°C, respectively). This was consistent with the seafloor observations of the gas bubbles, which consisted mostly of CH$_4$.

Except for CH$_4$, however, the major dissolved components (Na$^+$, K$^+$, Mg$^{2+}$, Cl$^-$, and SO$_4^{2-}$) in the bottom fluid samples showed neither enrichment nor depletion in comparison with seawater. If the site would be an active seeping site of some liquid material from deep sub-seafloor, it is difficult to assume such liquid having little anomalies in the major chemical components compared to seawater. The seeping material seems to be limited to gas bubbles and liquid a few amount in the knoll. That is, simple upward migration of the gases from sub-seafloor gas reservoir and mixing into bottom seawater and/or shallow pore water might be more probable for the mechanism to explain the CH$_4$-enrichment in the bottom fluid samples.

**Effluent plume**

We found clear enrichment of CH$_4$ in the water column at depths between 536 m and 644 m, the potential densities (expressed as specific gravity anomalies) being almost uniform at 28–30 kg/m$^3$ (Fig. 6). On the other hand, we did not find enrichment of CH$_4$ at depths less than 500 m. The maximum enrichment was 82.0 nmol/kg at a depth of 615 m for station 01-0 (bottom depth of 640 m), located just above the gas seeping site (Fig. 2), while the background level was around 1 nmol/kg at that depth (Fig. 6). In addition, we found smaller but clear anomalies at depths of 600–630 m for stations 01-1 and 01-2, both of which were about 3 km horizontally from the gas seeping site.

The depths of the seafloor at stations 01-1 and 01-2 were ca. 1050 and 870 m, respectively and thus it is unlikely that the vertical transport of CH$_4$ (or CH$_4$-enriched fluid) emitted from adjacent seafloor was the cause of CH$_4$ enrichment at stations 01-1 and 01-2. The lateral transport of the CH$_4$ plume from station 01-0 (gas seeping site) is more plausible.

The vertical transport of CH$_4$ in station 01-0 from the adjacent gas seeping site was limited to heights of less...
than 140 m from the seafloor, probably owing to complete dissolution of the gas bubbles to that height. After dissolution, the plume spread horizontally along with the surface of equal density of the plume, while the concentrations reduced through either dilution or oxidation in the oxic, low-CH₄ water column.

**DISCUSSION**

*Initial generation of hydrocarbons in the bubbles*

Both chemical and isotopic compositions of gaseous alkanes (C₁-C₄) are useful for inferring the origin of hydrocarbons. Specifically, we can differentiate thermogenic hydrocarbons (generated by thermal degradation of organic matter) from biogenic hydrocarbons (generated by microbial degradation of organic matter) by plotting the stable carbon isotopic compositions of CH₄ (δ¹³C₁) vs. the light gas composition (C₁/(C₂+C₃)) on a widely used conventional diagram (Bernard et al., 1977). The application of this diagram in the identification of surface gas seepage, however, provides misleading information for gases that underwent later alteration, such as oxidation of biogenic gas or molecular separation during migration (Tsunogai et al., 2002a).

In Fig. 3, the C₁/(C₂+C₃) ratio of the Kuroshima bubble samples is plotted as a function of δ¹³C for CH₄ (δ¹³CC₄). Approximate source regions of both thermogenic and biogenic hydrocarbons are also plotted together with a hypothetical mixing line between them having representative compositions: δ¹³CC₄ = -45‰VPDB and C₁/(C₂+C₃) = 10 for thermogenic hydrocarbons and δ¹³CC₄ = -80‰VPDB or -60‰VPDB and C₁/(C₂+C₃) = 10⁴ for biogenic hydrocarbons (Bernard et al., 1977). While the high C₁/(C₂+C₃) ratios suggest microbial generation, the ¹³C-enriched δ¹³C values of CH₄ (-40.1 ± 0.5‰VPDB) suggest thermogenesis. It is difficult to explain this contradiction through mixing between microbial and thermogenic hydrocarbons, as schematically shown by the theoretical mixing line. Rather, there was significant secondary alteration subsequent to generation.

One possible secondary process is the δ¹³C isotopic fractionation due to microbial oxidation of CH₄ under an aerobic/anaerobic condition. Tsunogai et al. (2002a) reported up to 50‰ ¹³C-enrichment in residual CH₄ through anaerobic microbial oxidation within surface marine sediments. The carbonate crusts including chimney-like structures found in the knoll also suggest anaerobic oxidation of CH₄ and subsequent carbonate precipitation following the reactions:

\[
\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O} \quad (4)
\]

\[
2\text{HCO}_3^- \rightarrow \text{CO}_2 + \text{CO}_3^{2-} + \text{H}_2\text{O} \quad (5)
\]

An aerobic oxidation of CH₄, however, results in the reduction of the C₁/(C₂+C₃) ratio in most cases (Whiticar et al., 1986), and thus it is difficult to explain the observed contradiction. Post-genesis heavy hydrocarbons from thermogenic hydrocarbons could explain the observed contradiction. Post-genesis heavy hydrocarbons through molecular fractionation is ubiquitous for natural hydrocarbons (Nagy, 1960; Price and Schoell, 1995; Mango, 2001; Snowdon, 2001). There is almost one order of magnitude less C₃ than C₂ (C₂/C₃ > 9) in the bubbles which suggests some molecular fractionation is responsible for the C₁-enrichment in hydrocarbons. Furthermore, the ¹³C-enrichment of C₃ (19.1‰VPDB), iso-C₄ (22.4‰VPDB), and n-C₄ (19.9‰VPDB) in comparison with C₂ (-28.3‰VPDB) and C₃ (n-C₃ + iso-C₃) (-28.0‰VPDB) suggests significant secondary alteration for the hydrocarbons. Regarding thermogenic hydrocarbons, the δ¹³C value for each hydrocarbon reflects the integrated kinetic isotope effect during the cleavage of chemical bonds from parental material. This begins with long-chain organic matter and thus smaller carbon number molecules tend to exhibit lower δ¹³C, with there being a reverse correlation with the reciprocal of the carbon number (Chung et al., 1988; Mango and Elrod, 1999; Du et al., 2003) as shown by the dotted arrow in Fig. 5. The δ¹³C values of C₁ and n-C₄ in the bubbles are larger than the values expected for C₄ and n-C₄ produced from sedimentary organic matter (~20 to -30‰VPDB) and also the value for coexisting C₃ (28.0‰VPDB) through thermogenic decomposition (Chung et al., 1988; Mango and Elrod, 1999; Du et al., 2003) or even microbial processes (Oremland et al., 1988; Taylor et al., 2000; Nakagawa et al., 2003). Hence, removal processes for the heavier hydrocarbons accompanying substantial isotopic fractionation must be highly probable for the C₁-enrichment in the hydrocarbons in the bubbles.

One possible molecular and isotopic fractionation process is fractional crystallization during hydrate formation. Gas hydrate is an ice-like crystalline mineral in which hydrocarbons are held within rigid cages of water molecules. While the structure I is usually pure CH₄, Ca + CO₃²⁻ → CaCO₃.
(Kvenvolden, 1995), structure II and structure H include C_{2}-C_{4} hydrocarbons as significant components (Sloan, 1998). The molecular distribution of the bubbles could be consistent with those substracting the hydrocarbons forming the structure II/H hydrate from the original thermogenic hydrocarbons enriched in C_{2}-C_{4} hydrocarbons, as a consequence of active gas hydrate crystallization during migration in the sub-seafloor. Such hydrate crystallization is significant in the knoll and accompanied by significant carbon isotopic fractionation, the residual bubbles would be CH_{4}-enriched relative to the C_{2}-C_{4} hydrocarbons and the C_{2}-C_{4} hydrocarbons would be \textsuperscript{13}C-enriched relative to the initial hydrocarbons. In previous field observations, however, little carbon isotopic fractionation was found during crystallization of gas hydrate, including C_{2}-C_{4} hydrocarbons (Brooks et al., 1986; Kennicutt et al., 1988). It is difficult to explain the observed \textsuperscript{13}C-enrichment for C_{1} and \nu_{C_{1}} in the bubbles as a consequence of the fractional crystallization during hydrate formation, and thus we should assume alternative processes.

An alternative process for explaining both molecular and isotopic fractionations is the fractional decomposition of hydrocarbons through some sub-seafloor microbial activity. James and Burns (1984) found that C_{1} and C_{3} alkanes were significantly \textsuperscript{13}C-enriched compared with other hydrocarbons in deep subsurface hydrocarbon reservoirs, which they attributed to selective microbial consumption of C_{1} and C_{3} alkanes. In Fig. 5, D\textsuperscript{13}C values for the hydrocarbons in the bubbles are plotted as a function of the reciprocal of each carbon number, together with values for the Sirus-1 Exmouth Plateau, offshore Australia, for which significant secondary alteration has been suggested (James and Burns, 1984). The alteration would probably be due to anaerobic microorganisms (Perry, 1980; Stephens and Dalton, 1986; Ashraf et al., 1994; Head et al., 2003). The two sets of results coincide strikingly well. Regarding the microbial processes for these deep subsurface hydrocarbons, preferential degradation of C_{1} followed by that of C_{3} and then that of C_{2} has also been noted (Boreham et al., 2001) with particularly high levels of biodegradation in lower-temperature (<60°C) reservoirs (Wenger et al., 2002). In addition, Strapoc et al. (2007) found kinetic isotope fractionation in propane presumably produced through anaerobic microbial consumption. The kinetic isotope fractionation during aerobic microbial consumption of C_{2}-C_{4} hydrocarbons has been quantified in laboratory incubation experiments as well (Kinnaman et al., 2007; Bouchard et al., 2008). Thus, microbial consumption of C_{1} (significantly), C_{3} (moderately), and C_{2} (partly) in thermogenic hydrocarbons could explain the observed molecular and isotopic fractionations of hydrocarbons.

To verify this hypothesis quantitatively, we estimated the initial C_{i}/(C_{2}+C_{3}) (the C_{i}/(C_{2}+C_{3}) prior to the microbial alteration) by correcting the microbial alteration in the concentrations and D\textsuperscript{13}C values of C_{2}-C_{4} hydrocarbons, assuming (1) the microbial alteration was limited to C_{2}-C_{4} hydrocarbons, so that the observed concentrations and the D\textsuperscript{13}C values in the bubbles corresponds to those of the initial for methane (103 ± 3 vol% and –40.1‰) and C_{4} (0.4 ppmv and –28.0‰), (2) the initial \textsuperscript{13}C values of C_{2}, C_{3}, and C_{4} were –33.0‰, –30.5‰, and –29.0‰, respectively, showing a reverse correlation with the reciprocal of carbon number in the initial C_{2}-C_{4} hydrocarbons, (3) the KIE (–1000(1/\alpha – 1)) in carbon isotopes of C_{2} during the microbial alteration was 3 ± 1, which corresponds to the KIE during the atmospheric oxidation of C_{2} (Tsunogai et al., 1999), (4) the KIEs in carbon isotopes of C_{2} and C_{3} during the microbial alteration were 2 ± 0.7 and 1.5 ± 0.5, respectively, which corresponds to 2/3 and 2/4 of the KIE of C_{2}, due to the dilution effect of KIEs by unreacted carbon in a molecule (Tsunogai et al., 1999). The assumed KIE for C_{3} corresponds well to that determined by the field observation (Strapoc et al., 2007).

Under these assumptions, we estimated the initial C_{i}/(C_{2}+C_{3}) from the concentrations and D\textsuperscript{13}C values of C_{2}-C_{4} hydrocarbons in the bubbles, by correcting the microbial alteration applying the Rayleigh equation (7) (Coleman et al., 1982) to each hydrocarbon.

\[
D\textsuperscript{13}C_{i} = D\textsuperscript{13}C_{0} = 1000(1/\alpha – 1)\ln(C_{i}/C_{0}),
\]

where C_{i} and C_{0} were the initial and final concentrations, respectively, and D\textsuperscript{13}C_{i} and D\textsuperscript{13}C_{0} were the initial and final D\textsuperscript{13}C values, respectively. The estimated initial concentrations of C_{2}-C_{4} hydrocarbons corresponds to the initial C_{i}/(C_{2}+C_{3}) of 235 (median), 19 (minimum) and 612 (maximum). All the initial C_{i}/(C_{2}+C_{3}) ratios correspond to that of the thermogenic hydrocarbons (Fig. 3).

In conclusion, microbial consumption of C_{1} (significantly), C_{3} (moderately), and C_{2} (partly) in thermogenic hydrocarbons can explain the observed molecular and isotopic fractionations of hydrocarbons, by increasing the C_{i}/(C_{2}+C_{3}) and D\textsuperscript{13}C values for C_{3} (significantly), C_{4} (moderately), and C_{2} (partly) of residual hydrocarbons.

**Evidence for the contribution of geothermal heat**

The thermogenic origin of the hydrocarbons is also supported by the \textsuperscript{3}He/\textsuperscript{4}He ratio of coexisting helium. The contribution of primordial, \textsuperscript{3}He-enriched helium has been widely recognized in thermogenic natural gases in subduction zones, reflecting the contribution of mantle heat in converting organic matter to hydrocarbons thermogenically (Poreda et al., 1988).

In Fig. 4, the measured \textsuperscript{4}He/\textsuperscript{3}He ratios of helium in the bubble samples are plotted as a function of \textsuperscript{4}He/\textsuperscript{20}Ne.
ratios (solid circles) together with those for the mantle (8R₄) where 8R₄ denotes the ³He/²⁰Ne ratio of 1.4 × 10⁶ (Lupton, 1983), continental crust (about 0.02R₄) (Lupton, 1983; Mamyrin and Tolstikhin, 1984) and sediments (the pore gases in the sedimentary layer) around Japanese islands (Deep Sea Drilling Project sites 583D, 583F, and 584; Sano and Wakita, 1987) (open triangles). To exclude samples contaminated by air to a significant extent, the data for sediments are limited to sediments having ³He/²⁰Ne ratios exceeding 0.5. In addition, bold lines for the theoretical mixing with seawater (air saturated seawater; 1R₄) are presented for the mantle and crust. Similarly, the mixing with seawater is presented for both the sedimentary pore gases and Kuroshima bubbles by dotted lines. By extrapolating the dotted mixing lines to the region with higher ³He/²⁰Ne ratios, we can obtain the endmember ³He/²⁰Ne ratios for the bubbles and sediments as 0.44R₄ and 0.15R₄ respectively, through removing seawater contaminants from the samples, assuming all ²⁰Ne in the samples was derived from the seawater.

While the endmember ³He/²⁰Ne ratio for the bubbles is lower than the ratios for the mantle or seawater, it is much higher than the ratio for the crust or sediments. It is difficult to explain the composition of Kuroshima bubbles merely by the in situ contribution of ³He-enriched radiogenic helium in the crust/sediments. Although the mixing of the seawater components with the crust or sediments could produce ³He/²⁰Ne ratios similar to those for the bubbles, it is impossible to explain the high ³He/²⁰Ne ratio of the bubbles through the mixing of seawater with sediments or crust as schematically shown in Fig. 4. Thus, there must be a contribution of mantle helium to the hydrocarbons in the bubbles, and thus thermogenic generation due to the contribution of geothermal heat is highly probable for the initial generation of hydrocarbons in the bubbles.

Comparison with the nearby natural gas seepages

The gas bubbles exhibit chemical and isotopic compositions that are similar to those of shallow-seafloor gas bubbles at the Taketomi spring. The Taketomi submarine spring (10–30 m depth) is 20 km northwest of the knoll and on the same forearc seafloor of the southern Ryukyu arc (Fig. 1). Gas bubbles composed mostly of CH₄ (70–80%) are discharged together with hot water having a temperature of up to 40–60°C (Kaneshima et al., 1983; Oomori, 1987; Oomori et al., 1993; Hirayama et al., 2007). The δ¹³C values of C₁ (−30 to −40‰VPDB), the C₂/C₁ ratios of 3000–5000, the C₂/C₁ ratios of 10, the ³He/²⁰Ne ratios of 0.7–0.9R₄, and the ³He/²⁰Ne ratios of 80–200 (Oomori et al., 1993) of the gas bubbles are comparable to values for Kuroshima Knoll. The Late Miocene Yaeyama Group, which includes coal and volcanic products, is assumed to be the source rock of the hydrocarbons in the Taketomi spring (Kaneshima et al., 1983). Similar ³He-enriched helium have been reported for subaerial natural gas seepages in western Taiwan (Sano et al., 1986; Poreda et al., 1988; Yang et al., 2003) accompanying thermogenic hydrocarbons (Lu and Lin, 1986; Yang et al., 2004; You et al., 2004), most of which originated from the Miocene Mushan and Wuchishan Formations (Lu and Lin, 1986). The contribution of mantle heat and generation of hydrocarbons through the thermocatalytic decomposition of organic matter is ubiquitous in the Miocene age of the area.

The surface of Kuroshima Knoll is covered mostly by the sedimentary rocks of the Pliocene Shimajiri Group (Matsumoto et al., 1998; Takeuchi et al., 2007). The sedimentary rocks of Miocene Yaeyama Group, however, usually underlies the Shimajiri Group in the southern Ryukyu islands (Tsuburaya and Sato, 1985). Although we could not find the Miocene Yaeyama Group on the surface, we can reasonably assume the Miocene Yaeyama Group exists in a deeper layer beneath the seafloor and it is highly probable that this group is the source rock of the gas bubbles in Kuroshima Knoll. Because the southern Ryukyu forearc area can be characterized by active volcanic activity in the Miocene (Kawano and Ueda, 1966; Monden, 1968; Tiba and Saito, 1974), both the initial generation in the Miocene and post-genesis storage until recent times in the group are consistent with our geochemical findings for the hydrocarbons of Kuroshima Knoll, such as (1) the contribution of geothermal heat to the initial generation so as to accumulate ³He-enriched mantle helium and (2) long storage in a deep crustal reservoir so as to reduce C₃–C₄ hydrocarbons, as presented in the previous sections.

Sub-seafloor anaerobic oxidation of methane

In comparison with the δ¹³C values of CH₄ in the bubbles (−40.1 ± 0.5‰VPDB), the values for bottom fluid samples show a substantial enrichment in ¹³C; +4% enrichment on average and +8‰ enrichment at maximum (Table 2). In addition, the lowermost δ¹³C value (−40.4‰VPDB) of CH₄ in the bottom fluid samples corresponds to values for the bubbles. It is unlikely there is a contribution of CH₄ from ambient water as for the cause of the δ¹³C variation because the concentrations of CH₄ in the bottom fluid (Table 2) were 10³–10⁶ times those in ambient bottom water (Fig. 6). We conclude that kinetic microbial oxidation of CH₄ must be responsible for the deviation: that is, CH₄ in the bottom fluid samples is the residue of CH₄ after the oxidation.

If the oxidation occurred only in the aerobic water column after venting into the water column, there should be ¹³C-enrichment in accordance with the depletion in the concentration. However, we could not find a clear correlation between the concentrations and δ¹³C values
of CH$_4$ in the bottom fluid samples (Table 2). Furthermore, while the samples D1356W2, D1356W3, and D1357W3 had concentrations $10^7$ times that of ambient seawater (almost to saturation), the $\delta^{13}$C values indicated a clear $^{13}$C-enrichment in comparison with the bubbles. It is unlikely such rapid oxidation in the water column had progressed in the fluid before dilution with the surrounding ambient water. We conclude that the majority of the oxidation progressed under the seafloor. This is in agreement with the observation by Inagaki et al. (2004), who observed $^{13}$C-enrichment of CH$_4$ up to 20‰ within pore water of the seeping site. The CH$_4$ in the liquid samples contained the residual CH$_4$ oxidized anaerobically during diffusive migration in the sedimentary layer through the reaction (4) (Kulm et al., 1986; Tsunogai et al., 2002a).

Because the reaction requires the mixing of seawater SO$_4^{2-}$ with CH$_4$, it might be limited to a thin surface layer, probably less than 1 meter thick. By reducing the alkalinity of the mixture, carbonates have precipitated in the knoll as observed at another cold seepage site (Kulm and Suess, 1990). On the other hand, the observed homogenous $\delta^{13}$C values of CH$_4$ in the bubbles suggest that they had not undergone anaerobic oxidation, probably owing to the rapid, advective migration of the bubbles in the sedimentary layer. The trace concentration of CO$_2$ in the bubbles also supports that there was minimum oxidation of the bubbles prior to seepage.

**Contribution of gas hydrate dissociation**

As already discussed, the contribution of gases derived from the dissociation of sub-seafloor methane hydrate is not required to explain the chemical and isotopic compositions of hydrocarbons in the knoll as observed at another cold seepage site where the dissociation of hydrate has been assumed (Kulm et al., 1986; Tsunogai et al., 2002a). However, it is difficult to explain the helium enrichment in the bubbles derived from hydrate because helium is rarely incorporated into the structure of natural methane hydrate (Dickens and Kennedy, 2000; Winckler et al., 2002). In the case of the pure natural gas hydrates taken from Hydrate Ridge, Cascadia Margin, for instance, the $^4$He/CH$_4$ ratios were from 0.0003 to 0.009 ppmv (Winckler et al., 2002), while the ratios for the Kuroshima bubbles were more than 10 ppmv. The dissociation of methane hydrate cannot be the only source of the bubbles in the knoll. Thus, if we persist with the contribution of methane hydrate, we must assume additional He-enriched gases for the source of the bubbles, which seems unrealistic for the area. Because the $^4$He/CH$_4$ ratios in the Kuroshima bubbles can be classified into the representative $^4$He/CH$_4$ ratios of thermogenic natural gases in and around Japan islands (Wakita and Sano, 1983; Urabe et al., 1985; Poreda et al., 1988), direct leakage of hydrocarbons from sub-seafloor gas reservoirs to seafloor is the most probable process to explain the origin of the bubbles in Kuroshima Knoll.

Submarine massive hydrate is ubiquitous in the deep ocean floor around the world (Brooks et al., 1986; Suess et al., 1999). Because hydrate decomposes when the temperature rises, decomposition of sub-seafloor methane hydrate has been assumed as the source of seafloor discharges of CH$_4$ bubbles at many sites of the world ocean, as presented for the case of the Kuroshima Knoll in this study. Both the present progression of global climate change and the findings of the massive deposits of methane hydrate on the seafloor at active CH$_4$ seeping sites seem to support the assumption. Most of the submarine massive hydrates, however, seems to be stable at present (Sassen et al., 2001). In addition, methane hydrate can be produced as a result of natural CH$_4$ seeping activity on the seafloor. Regarding the origin of the seafloor seeping bubbles, we also need to take into account other pos-
gas seeping from Kuroshima Knoll. This is consistent with observations of other bubbles seeping from seafloor (Suess et al., 2001). Because the depth is substantially deeper than the maximum mixed layer depth (ca. 100 m) in the area, direct input into atmosphere is negligible for the gas seeping from Kuroshima Knoll.

To clarify whether the CH4 decrease in the plume can be explained by simple dilution by the background seawater, the measured δ13C values of CH4 in the plume are plotted against the reciprocal of the CH4 concentration for the samples taken within the plumes (column water samples taken from 500 to 700 m depths) (Fig. 7). The least-squares fitted line for the plume samples is also shown in the figure. The linear correlation suggests that simple mixing between the seeping CH4 and CH4 in background seawater is the main process determining both the concentration and δ13C of CH4 in the plume, and the δ13C value of seeping CH4 is uniform during the mixing (Tsunogai et al., 1998, 2000, 2005). That is, aerobic microbial oxidation in the oxic water column is negligible for CH4 in the plume, and thus CH4 is diluted rapidly by eddy diffusion rather than oxidation in the water column. In conclusion, all the rising bubbles at Kuroshima Knoll have dissolved within 140 m of the seafloor. After dissolution, the plume spreads horizontally along with the surface of equal density in the water column, while the concentrations decrease through dilution by eddy diffusion, rather than by oxidation.

Quantification of sub-seafloor anaerobic oxidation

The intercept of the least-squares fitted line for the plume samples in Fig. 7 corresponds to the end-member δ13C value of CH4 seeping from the knoll (Tsunogai et al., 1998), which can be considered the weighted average δ13C value of CH4 emitted from the knoll to the water column. Assuming the δ13C values of the bubbles (−40.1‰VPDB) represent the initial δ13C of CH4 prior to sub-seafloor anaerobic oxidation, we can evaluate the extent of the sub-seafloor anaerobic microbial oxidation quantitatively using the discrepancy between the weighted average δ13C value (−38.6‰VPDB) and the δ13C values of the bubbles because the discrepancy (−2.5‰VPDB) corresponds to the average extent of the progress of the sub-seafloor anaerobic oxidation of CH4 at Kuroshima Knoll.

Assuming the kinetic isotope effect due to the anaerobic microbial CH4 oxidation $\alpha (k_2/k_1)$ to be 1.008 (Tsunogai et al., 2002a), the fraction of residual CH4 can be calculated from the δ13C variation using the Rayleigh equation (7). Using this equation, we estimated the average $C_f/C_0$ to be 0.82. Thus, about 20% of CH4 has been oxidized before seeping into the water column.

Conclusions

Most portions of the hydrocarbons in the Kuroshima Knoll bubbles were supplied directly from deep gas reservoirs produced by thermogenic processes in the Miocene. The hydrocarbons, especially C3- and C4-, had been fractionated both chemically and isotopically through microbial degradation during the storage in the gas reservoir. In addition, the anaerobic oxidation of CH4 within shallow sediments just beneath the seafloor removes about 20% of CH4 on average, until seepage into ocean water column. After the seepage, all rising bubbles dissolved into the ocean water column within 140 m of the seafloor. After the dissolution, the plume spreads horizontally along with the surface of equal density in the water column, while the concentrations decrease through dilution by eddy diffusion, rather than by oxidation.

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